An Investigation of Variables in a Fecal Flotation Technique

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ABSTRACT

Several variables in a standard vial fecal gravitational flotation technique were investigated. These were the specific gravity of the sodium nitrate flotation solution, duration of flotation and mesh sizes of strainers. The number of eggs which floated and adhered to a coverslip were counted and estimates of the number of eggs remaining in the strained fecal suspension and in the feces trapped on the strainer were made. Eggs from hookworms, Trichuris vulpis and Toxocara canis in feces from dogs, Nematodirus spp. from sheep and Parascaris equorum from horses floated equally well in solutions with specific gravities (SpGr) ranging from 1.22-1.38. Taenia spp. from dogs had a slightly narrower range (SpGr 1.27-1.38) for best recovery. Eggs from Haemonchus contortus from sheep appeared to float best between SpGr 1.22-1.32. Strongyles from one horse floated best with SpGr 1.27-1.32 and from another with SpGr 1.11-1.38. Coccidial oocysts from sheep floated best in a narrow range of SpGr from 1.22-1.27. However, as the SpGr of the solution was increased the recognition of eggs under the coverslip was increasingly difficult and especially so at SpGr 1.38 with sheep feces. This was due to the increasing amount of debris and the more rapid formation of crystals with evaporation with solutions of higher SpGr. It appeared, therefore, that solutions with SpGr of 1.22-1.35 would be best for routine laboratory use. At specific gravity 1.27, there appeared to be no difference in the number of eggs recovered for a four, eight and 12 min flotation period.

Only 3-7% of the eggs in 4 g of feces were counted under the coverslip. This poor efficacy resulted first because aproximately 50% of the eggs were trapped in the feces and retained on the strainer. Secondly, only one half of the strained fecal suspension, containing approximately 25% of the eggs, was placed in the vial for examination. Thirdly, of those eggs in the vial only 16-29% were counted under the coverslip. When the second half of the strained fecal suspension was placed in another vial, the amount of debris and air bubbles adhering to the coverslip was much less than that for the first vial. Egg counts for both vials appeared similar and it may be that when debris is excessive the fecal examination should involve counts from a second vial. The use of strainers finer than the standard tea strainer and the addition of minimal amounts of detergent did not increase the egg count.

RÉSUMÉ

Cette expérience consistait à étudier trois variantes d'une technique de flottation fécale standard, par gravité, à savoir: la gravité spécifique de la solution de nitrate soude, la durée de la flottation et les dimensions des pores des tamis. On

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détermina le nombre d'oeufs qui flottèrent et adhérèrent à une lamelle, ainsi que le nombre approximatif de ceux qui demeurèrent dans la suspension fécale tamisée et dans les fèces retenues par le tamis. Les oeufs de vers à crochets, de Trichuris vulpis et de Toxocara canis du chien, ceux de Nematodirus spp. du mouton et de Parascaris equorum du cheval, flottèrent tout aussi bien dans des solutions dont la gravité spécifique variait de 1.22 à 1.38, tandis que ceux des Taenia spp. du chien flottaient le mieux lorsque la gravité spécifique variait de 1.27 à 1.38 et que ceux de Haemonchus contortus du mouton flottaient aussi le mieux lorsque la gravité spécifique variait de 1.22 à 1.32. Les oeufs des strongles d'un cheval flottèrent le mieux, à une gravité spécifique de 1.27 à 1.32; ceux d'un autre cheval flottèrent par ailleurs de façon satisfaisante, à une gravité spécifique de 1.11 à 1.38. Les oocystes des coccidies ovines flottèrent le mieux, lorsque la gravité spécifique ne variait que de 1.22 à 1.27. À mesure qu'augmentait la gravité spécifique de la solution de flottation, l'identification des oeufs qui adhéraient à la lamelle devint toutefois de plus en plus difficile, surtout lorsqu'elle se situait à 1.38 et qu'on travaillait avec des fèces de moutons. Cette difficulté résultait d'une augmentation de la quantité de débris et de la formation plus rapide de cristaux, à la suite de l'évaporation des solutions de densité spécifique plus élevée. Les solutions dont la gravité spécifique varie de 1.22 à 1.35 s'avérèrent par conséquent les meilleures à utiliser couramment, au laboratoire. À la gravité spécifique de 1.27, on ne nota aucune différence dans le nombre d'oeufs recouvrés après quatre, huit, ou 12 minutes de flottation.

L'examen d'une échantillon de 4 g de fèces se solda par le dénombrement de seulement 3 à 7% des oeufs qu'il contenait. Ce piètre résultat s'expliquait ainsi: environ 50% de ces oeufs se trouvèrent emprisonnés dans les fèces ou retenus par le tamis; on ne soumit à la flottation que la moitié de la suspension fécale tamisée, laquelle contenait environ 25% des oeufs; on ne dénombra sous la lamelle qu'environ 16-29% des oeufs que contenait le tube. Lorsqu'on vida dans un autre tube l'autre moitié de la suspension fécale tamisée, beaucoup moins de débris et de bulles d'air adhérèrent à la lamelle. Le nombre d'oeufs se révéla comparable, dans chacun des deux tubes; l'examen fécal devrait sans doute comporter un comptage à partir d'un second tube, lorsque l'échantillon recèle une quantité particulièrement élevée de débris. L'utilisation de tamis dont les pores sont plus petites que celles des tamis à thé standards et l'addition d'une quantité infime de détergent n'augmentèrent pas le nombre d'oeufs qu'on pouvait compter.

INTRODUCTION

"Feces puddling is by no means an exact science" as Georgi (1) has pointed out and the examination of fecal material for ova, cysts and larvae of parasitic helminths and protozoa can be performed in several different ways (1, 5). In 1949, Kingscote at the Ontario Veterinary College incorporated the sputum vial in a gravitational flotation technique and an arbitrary system to quantify the results (4) was introduced by McCraw (McCraw, B.M., personal communication). Koutz (3) has shown that fecal techniques utilizing centrifugal force are more efficient in recovering parasite eggs than those using gravitational force. Nevertheless, partly because there is no relationship between numbers of eggs and numbers of parasites in a host (2), and certainly due to the simplicity and low cost of the procedure, the semiquantitative Sputum Vial Fecal Flotation technique has found wide use in diagnostic laboratories throughout Canada. However, there is a lack of critical work on gravitational flotation techniques. In this investigation several variables, specific gravity of sodium nitrate, duration of flotation and mesh size of strainers as used in the technique were studied.

MATERIALS AND METHODS

To 4 g of feces from dogs, horses or sheep was added 60 mL of a solution of sodium nitrate ($NaNO_3$) of known specific gravity (SpGr) and ranging from 1.22-1.38. Prior to the addition of $NaNO_3$, tapeworm segments were removed from the feces of one dog, ground with physio-

logical saline and the suspension strained and centrifuged. After the supernatant was decanted, the precipitate was resuspended in saline and 0.3 mL of the egg suspension was added to 4 g of dog feces.

After thorough mixing of feces and $NaNO_3$, the mixture was strained using a tea strainer. In one procedure, stainless steel mesh[1] with sizes of 500, 350 and 250 μ square were used for straining. The strained fecal fluid was shaken thoroughly and poured into a sputum vial (Vial A) allowing the meniscus to project above the rim of the vial on which was placed a coverslip. After a period (flotation period), ranging from four to 12 min, the coverslip was removed vertically, with the aid of coverglass forceps, placed on a glass slide and the adhering fluid examined microscopically. The eggs under the coverslip and in the fluid in the adjoining area were counted (coverslip count). After Vial A was poured, there remained sufficient fluid to fill a second sputum vial (Vial B) and in some instances a coverslip count for Vial B was made. The volume of the sputum vials was 24 mL.

In one procedure, after Vials A or B were filled with the strained fecal suspension, the latter was poured into a beaker and shaken. One mL was withdrawn, using a 1 mL tuberculin syringe with the barrel cut off at the zero mark, and placed in a counting chamber (1). The number of eggs counted was multiplied by 24. In that procedure, the fecal material retained on the strainer (fecal discard) was placed in a graduated cylinder and $NaNO_3$ solution added to bring the suspension up to 48 mL. After the suspension was mixed, 1 mL was withdrawn, placed in the counting chamber and the number of eggs counted and multiplied by 48. In another procedure, after the coverslip was removed from a vial, a 1 mL pipette was inserted and 1 mL of fluid withdrawn from the top, middle and bottom of the vial. Each sample was placed in a counting chamber and the number of eggs counted.

In one experiment, the amount of debris and air bubbles adhering to the coverslip in Vials A and B were measured using an arbitrary scale of ½ to 6 where 6 was maximum. The assessment was made before and after removal of the coverslip from the vial. In another experiment, 0.1 or 1 mL of a 25% solution of Palmolive[2], Joy[3], Sweetheart[4], sodium dodecosulfate, Triton[5] and Alconox[6] were added to sheep feces and $NaNO_3$ solution. In this procedure, the number of eggs in 1 mL of the fecal discard fluid was counted as described previously.

After square root transformations, the mean egg counts were analysed by analysis of variance and Tukey's multiple comparison tests (6).

RESULTS

Feces were collected from four dogs, two horses and five sheep. Feces from dogs contained eggs from one or more of *Toxocara canis, Taenia* spp., hookworms and *Trichuris vulpis*. Feces from horses contained strongyle eggs and one horse had additionally *Parascaris equorum* eggs. The feces from sheep contained eggs from one or more of *Haemonchus contortus*[7], *Nematodirus* spp., GIN[8] type and coccidia.

Coverslip counts for samples processed with different SpGr of $NaNO_3$ are shown in Table I. There was no difference when the SpGr ranged from 1.22-1.38 for *T. canis,* hookworms, *T. vulpis, Nematodirus* spp. and *P. equorum;* from 1.22-1.27 for coccidial oocysts; from 1.27-1.38 for *Taenia* spp. and from 1.11-1.32 for *H. contortus* and strongyles. However, strongyles from one horse floated equally well with SpGr from 1.11-1.38. At SpGr 1.11, *P. equorum*

[1]W.S. Tyler and Company, St. Catharines, Ontario.

[2]Colgate-Palmolive Limited, Toronto, Ontario.

[3]Procter & Gamble Company of Canada Limited, Toronto, Ontario.

[4]Pirex Corporation Ltd., Weston, Ontario.

[5]Canlab Chemicals, Toronto, Ontario.

[6]Alconox Inc., New York, N.Y.

[7]The sheep were experimentally infected with H. contortus.

[8]GIN refers to those gastrointestinal nematodes in ruminants belonging to the families of Strongyloidea and Trichostrongyloidea, all of which produce an oval shaped thin-shelled egg and enclosing an embryo in the morula stage.

TABLE I. $\sqrt{\text{Mean Number of Parasite Eggs}}$ under Coverslips Removed after a Flotation Period of Eight Minutes from Vials Containing Feces in $NaNO_3$ Solutions of Different Specific Gravities. For Each Host all Counts were made from a Single Collection of Feces

Host	Parasite	Specific Gravity 1.38	1.35	1.32	1.27	1.22	1.11	N^a	Standard Deviation
Dog 1	*Toxocara canis*	3.3^a	3.5^a	2.4^{ab}	2.7^{ab}	2.5^{ab}	1.0^b	3	0.67
Dog 2	*Toxocara canis*	6.5^a	6.7^a	7.8^a	6.8^a	6.4^a	2.8^b	3	1.13
Dog 3	*Taenia* spp.	12.5^a	10.9^a	10.5^a	5.6^{ab}	1.6^b	1.8^b	3	2.69
Dog 4	Hookworms	15.8^a	21.6^a	22.8^a	22.8^a	24.1^a	15.0^a	3	3.84
	Trichuris vulpis	10.8^a	8.4^a	9.5^a	8.5^a	8.4^a	0.7^b	3	1.09
Sheep 1	*Haemonchus contortus*	11.6^a	13.4^{ab}	19.3^{abc}	25.8^{bc}	24.3^{abc}	26.6^c	3	4.68
Sheep 2	*Nematodirus* spp.	4.3^a	4.3^a	4.7^a	6.2^a	5.1^a		2	0.77
	Coccidia	12.8^a	12.8^a	15.8^{ab}	20.6^{bc}	23.2^c		2	1.59
Horse 1	Strongyles	9.3^a	13.9^a	12.7^a	13.8^a	16.4^a	10.9^a	2	3.66
Horse 2	Strongyles	16.0^a	16.7^a	19.3^{ab}	21.9^{ab}	24.4^b	26.3^b	3	2.60
	Parascaris equorum	6.3^a	6.3^a	6.7^a	5.4^a	4.7^a	0^b	3	1.04

N^a = Number of observations per mean
^{abc} = Means with different superscripts are significantly different at $\alpha = 0.05$

TABLE II. $\sqrt{\text{Mean Number of Parasite Eggs}}$ under Coverslips removed from Vials Containing Feces in $NaNO_3$ Solution (SpGr 1.27) and after Different Flotation Periods. For each Host, all Counts were made from a Single Collection of Feces

Host	Parasite	Duration of Flotation (Min) 2	4	8	12	N^a	Standard Deviation
Sheep 2	*Nematodirus* spp.	6.3^a	5.2^b	6.3^a	6.0^{ab}	3	0.39
	GIN	14.0^a	18.5^b			3	1.86
	Coccidia	15.2^a	17.0^a	22.5^b	26.5^b	3	1.54
Sheep 3	*Haemonchus contortus*		27.8^{ab}	33.0^a	25.0^b	3	2.95
Horse 1	Strongyles		16.3^a	15.4^b	15.7^{ab}	2	1.18
Horse 2	Strongyles		18.3^a	20.7^a	21.2^a	3	1.90
	Parascaris equorum		6.1^a	6.3^a	6.2^a	3	0.96

N^a = Number of observations per mean
^{ab} = Means with different superscripts are significantly different at $\alpha = 0.05$

was not recovered and there were few *T. vulpis*. There was considerable variation in the amount of crystallization and fecal debris appearing beneath the coverslip with solutions of different SpGr. With a SpGr of 1.38 the amount of crystallization was quite high when the duration of microscopic examination was greater than five min. With a SpGr of 1.11 no crystallization occurred during a 30 min examination. At SpGr 1.11 the amount of debris was negligible but the amounts appeared to increase as the SpGr was increased.

Coverslip counts for different flotation periods for samples from sheep and horses with the SpGr of $NaNO_3$ at 1.27 are shown in Table II. In general, there appeared to be no difference between a four and 12 min flotation period for egg types investigated except coccidia which were more numerous at eight and 12 min. At SpGr 1.27, there appeared to be no difference in the amount of crystals forming between the four and 12 min periods.

The number of eggs in Vials A and B containing sheep feces with $NaNO_3$, SpGr 1.27 and before flotation adherence to a coverslip were similar and lower than in the fecal discard (Table III). If the egg counts are compared on the arithmetic scale it appeared that the fecal discard contained about twice as many eggs as Vials A or B. After a four min flotation period and removal of the coverslip, the mean number of eggs in 1 mL of fluid from the top and middle of a vial containing sheep feces in $NaNO_3$ (SpGr 1.27) were similar and lower than that from the bottom of the vial (Table IV).

When the amount of debris and air bubbles adhering to coverslips in Vials A and B containing sheep feces in $NaNO_3$ (SpGr 1.38) were compared after a four min flotation period, it appeared that Vial A

TABLE III. Mean (three replicates) Number of *Haemonchus contortus* Eggs found in Different Portions of a Suspension of Sheep Feces in $NaNO_3$ Solution (SpGr 1.27). For each Host Counts were made from a Single Collection of Feces

Host	Suspension	Mean Arithmetic	Mean $\sqrt{\text{Arithmetic}}$
Sheep 3	Vial A	8416	91.74[a]
	Vial B	6974	83.52[a]
	Fecal Discard	14047	118.52[b]
Sheep 5	Vial A	5000	70.71[a]
	Vial B	4896	69.97[a]
	Fecal Discard	13152	114.68[b]

[ab] = Means with different superscripts are significantly different at $\alpha = 0.05$

contained much larger amounts (Table V). The coverslip count data for this experiment were not analysed statistically, but it appeared that the counts for Vials A and B were similar. When strainers of different mesh sizes were used there appeared to be no difference in coverslip counts from sheep feces in $NaNO_3$ solution (SpGr 1.27) and after a four min flotation period (Table VI). The trial with minute quantities of detergents added to sheep feces was a preliminary one and did not appear to reduce the numbers of eggs in the fecal discard.

DISCUSSION

In an examination of the sputum vial fecal flotation technique which uses sodium nitrate as the flotation medium, most of the common parasite egg types from dogs, sheep and horses were recovered over a wide range of SpGr (from 1.11-1.38). Eggs from hookworms, *T. vulpis*, *T. canis* in feces from dogs, *Nematodirus* spp. from sheep and *P. equorum* from horses floated equally well in solutions with SpGr ranging from 1.22-1.38. *Taenia* spp. from dogs had a narrower range of SpGr (1.27-1.38) for best recovery. Eggs from *H. contortus* from sheep appeared to float best between SpGr 1.22-1.32. Strongyles from one horse floated best with SpGr 1.27-1.32 and from another with SpGr 1.11-1.38. Coccidial oocysts from sheep floated best in a narrow range of SpGr (from 1.22-1.27).

However as the SpGr of the $NaNO_3$ solution was increased, the recognition of eggs under the coverslip was increasingly difficult. There were two reasons for this. First, there appeared to be increasing amounts of fecal debris floating with increasing SpGr and this was especially marked with sheep fecal samples processed at SpGr 1.38. Secondly, with increasingly higher SpGr, crystallization appeared to occur more readily. Crystals were found around the coverslip both on the sputum vial and when the former was removed and placed on the glass slide and especially if the fecal debris under the coverslip was excessive. From these data and observations it would appear that for general purpose laboratory use, a solution of $NaNO_3$ with SpGr from 1.22 to 1.35 would be preferable. When a SpGr of 1.27 was used, there was no difference in the coverslip counts for most egg types for flotation periods of four, eight and 12 min.

The total number of eggs in Vials A and B were similar, but on the arithmetic scale there was twice that number trapped in the fecal discard. Therefore, approximately 25% of the eggs in 4 g of feces is contained in Vial A, the vial which is usually examined. After a four min flotation period and removal of the coverslip, the number of eggs at the top and middle

TABLE IV. Number of *Haemonchus contortus* Eggs in Various Portions of a Vial containing Sheep Feces in $NaNO_3$ Solution (SpGr 1.27) and after Removal of the Coverslip following a Four Minute Flotation Period. A Single Collection of Feces was used

Region	Replicate 1	Replicate 2	Replicate 3	$\sqrt{\text{Arithmetic}}$ Mean
Coverslip	2473	1157	1231	
Top 1 mL	119	138	209	12.4[a]
Middle 1 mL	119	213	218	13.4[a]
Bottom 1 mL	553	256	386	19.7[b]

[ab] = Means with different superscripts are significantly different at $\alpha = 0.05$

TABLE V. Amount of Debris and Air Bubbles, using a Scale of ½ to 6 where 6 was Maximum, and Number of GIN Eggs Adhering to Coverslips from Vials Containing Sheep Feces in $NaNO_3$ Solution (SpGr 1.38) and After a Four Minute Flotation Period. The Assessment was Performed Before and After the Coverslip was Removed from the Vial and Placed on a Glass Slide. Several Replicates were Performed on Each of Two Collections of Feces

Collection	Replicate	Coverslip Position	Vial A			Vial B		
			Debris	Bubbles	Eggs	Debris	Bubbles	Eggs
1	1	Vial	5	2		3	½	
		Glass slide	4	2	69	3	½	N[a]
	2	Vial	5	6		2	2½	
		Glass slide	4	5	N	2	2½	90
	3	Vial	6	6		3	½	
		Glass slide	6	5	65	2½	½	N
2	1	Vial	6	6		4	5	
		Glass slide	4	6	17	2	5	14
	2	Vial	5	6		3	3	
		Glass slide	5	6	31	3	3	43
	3	Vial	2	3		2	3	
		Glass slide	1	½	12	1	½	26

[a] Number of eggs not counted

TABLE VI. $\sqrt{\text{Mean Number of Parasite Eggs}}$ under Coverslips Removed from Vials Containing Feces in $NaNO_3$ Solution (SpGr 1.27) after a Four Minute Flotation Period and using Strainers of Different Mesh Sizes. For each Host all Counts were made from a Single Collection of Feces

Host	Parasite	Mesh Size (μ)			N^a	Standard Deviation
		500	350	250		
Sheep 4	*Haemonchus contortus*	30.2[a]	36.1[a]	36.0[a]	2	3.54
Sheep 5	*Haemonchus contortus*	29.7[a]	32.6[a]	33.1[a]	3	2.24
Horse 1	Strongyles	21.6[a]	19.2[b]	19.7[ab]	6	1.51
Horse 2	*Parascaris equorum*	6.7[a]	6.0[a]	6.3[a]	3	0.80

N^a = Number of observations per mean
[ab] = Means with different superscripts are significantly different at $\alpha = 0.05$

of the vial were similar and fewer than at the bottom. For a crude estimate of the number of eggs remaining in the vial one might assume that the number of eggs at top, middle and bottom of the vial were similar and multiply the arithmetic mean by the volume of fluid in the vial. If to this figure is added the coverslip count a crude estimate of the number of eggs in the vial before applying the coverslip can be made. If this is done for replicates 1, 2 and 3 in Table IV the total number of eggs would be 8537, 5811 and 7464 respectively. When the coverslip counts are compared with these values, the proportion of eggs retrieved with the coverslip would be 29, 20 and 16% respectively. Since the vial contained approximately 25% of the total number of eggs in a 4 g fecal sample, only 3-7% of the eggs were recoverable under the coverslip. The technique, therefore, is very inefficient. In the diagnostic laboratory precise measurements of the ingredients are not done and the error rate may be greater. It would be important, therefore, in such laboratories, to keep the proportions of feces and $NaNO_3$ solution constant to minimize that error.

Vials A and B may contain approximately equal numbers of eggs, but Vial A contained more debris and air bubbles beneath the coverslip. Although the strained suspension was well mixed and poured immediately, the numerous light particles still managed to float rapidly and escape into Vial A and made the detection of eggs difficult. The painstaking examination involved in such a process would be exceptional in a diagnostic laboratory and when debris is excessive it may be that the technique should be performed with Vial B in preference to Vial A. Further work may yet prove that the coverslip count from Vial B is higher than Vial A when debris is excessive and when there are higher egg counts than those studied here.

Attempts to increase the number of eggs which floated to the coverslip by using strainers of differing mesh sizes and by the addition of detergents and surface acting agents were unsuccessful. Strainers with a mesh size from 500 (the size of a tea strainer) to 250 μ square produced similar results. Strainers finer than 250 μ square may inhibit the passage of eggs. However, the work with detergents and surfactants needs to be studied further.

In these studies forceps were used to remove the coverslip from the vial. This procedure appeared to allow the adherence of a greater amount of fecal suspension to the coverslip when compared with removing it with one's fingers. The use of forceps has the added advantage of being more aesthetic and a more desirable public health procedure.

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